09/424347

Practitioner's Docket No. 49429

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CHAPTER II

TRANSMITTAL LETTER
TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/JP98/02171	18 May 1998	23 May 1997
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

ORGANIC ANION TRANSPORTER AND GENE CODING FOR THE SAME
TITLE OF INVENTION

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APPLICANTS

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

NOTE *To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1 492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.*

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. §1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 C.F.R. §1.8.*

NOTE: *Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 C.F.R. § 1.494(f).*

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

   a. [X] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

   b. [X] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

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2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
[ ]*	TOTAL CLAIMS	27 - 2	7	x $ 18.00 =	$126.00
	INDEPENDENT CLAIMS	3 -	0	x $ 78.00 =	
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + $260.00				$260.00
BASIC FEE**	[ ] U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: [ ] and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(2) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4)) .... $96.00 [ ] and the above requirements are not met (37 CFR 1.492(a)(1)) .... $670.00 [X] U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the USPTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: [ ] has been paid (37 CFR 1.492(a)(2)) .... $760.00 [ ] has not been paid (37 CFR 1.492(a)(3)) .... $970.00 [X] where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) $840.00				$840.00
	Total of above Calculations				= $1,226.00
SMALL ENTITY	Reduction by ½ for filing by small entity, if applicable. Affidavit must be filed. (note 37 CFR 1.9, 1.27, 1.28)				-
	Subtotal				$1,226.00
	Total National Fee				$1,226.00
	Fee for recording the enclosed assignment document $40.00 (37 CFR 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				$1,226.00

*See attached Preliminary Amendment Reducing the Number of Claims.

i. [X] A check in the amount of $1,226.00 to cover the above fees is enclosed.
ii. [ ] Please charge Account No. ___________ in the amount of $ ___________.
A duplicate copy of this sheet is enclosed.

**WARNING: *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. [X] A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: *Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.*

a. [X] is transmitted herewith (published as WO 98/53064).
b. [ ] is not required, as the application was filed with the United States Receiving Office.
c. [ ] has been transmitted
  i. [ ] by the International Bureau.
  Date of mailing of the application (from form PCT/IB/308): ___________.
  ii. [ ] by applicant on _______.
  Date

4. [X] A translation of the International application into the English language (35 U.S.C. 371(c)(2)):
a. [X] is transmitted herewith.
b. [ ] is not required as the application was filed in English.
c. [ ] was previously transmitted by applicant on ________________.
Date
d. [ ] will follow.

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

NOTE: *The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.*

a. [ ] are transmitted herewith.
b. [ ] have been transmitted
  i. [ ] by the International Bureau.
  Date of mailing of the amendment (from form PCT/IB/308): __________.

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ii. [ ] by applicant on ______.
Date

c. [X] have not been transmitted as

i. [X] applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210): 29 June 1999.

ii. [ ] the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):

a. [ ] is transmitted herewith.

b. [ ] is not required as the amendments were made in the English language.

c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)

[X] is transmitted herewith.

[ ] is not required as the application was filed with the United States Receiving Office.

8. [ ] Annex(es) to the international preliminary examination report

a. [ ] is/are transmitted herewith.

b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [ ] A translation of the annexes to the international preliminary examination report

a. [ ] is transmitted herewith.

b. [ ] is not required as the annexes are in the English language.

10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115

a. [ ] was previously submitted by applicant on ____________.
Date

b. [ ] is submitted herewith, and such oath or declaration

i. [ ] is attached to the application.

ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.

iii. [X] will follow.

Other document(s) or information included:

11. [X] An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. [X] is transmitted herewith.

b. [ ] has been transmitted by the International Bureau.
Date of mailing (from form PCT/IB/308): ________________.

c. [ ] is not required, as the application was searched by the United States International Searching Authority.

d. [ ] will be transmitted promptly upon request.
e. [ ] has been submitted by applicant on _________________.
Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:
a. [ ] is transmitted herewith.
Also transmitted herewith is/are:
[ ] Form PTO-1449 (PTO/SB/08A and 08B).
[ ] Copies of citations listed.
b. [X] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
c. [ ] was previously submitted by applicant on _________________.
Date

13. [ ] An assignment document is transmitted herewith for recording.

A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or[ ] FORM PTO 1595 is also attached.

14. [X] Additional documents:
a. [X] Copy of request (PCT/RO/101)
b. [X] International Publication No. WO 98/53964
i. [X] Specification, claims and drawing
ii. [ ] Front page only
c. [ ] Preliminary amendment (37 C.F.R. § 1.121)
d. [X] Other

Copy of the Demand Form

15. [X] The above checked items are being transmitted
a. [X] before 30 months from any claimed priority date.
b. [ ] after 30 months.

16. [ ] Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on
_____________, namely:

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AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: *"A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

NOTE: *"Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).*

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 04-1105 .

[X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

[X] 37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: *Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.*

[X] 37 C.F.R. 1.17 (application processing fees)
[X] 37 C.F.R. 1.17(a)(1)-(5)(extension fees pursuant to § 1.136(a).
[ ] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: *Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).*

NOTE: *37 C.F.R. 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.*

[ ] 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

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SIGNATURE OF PRACTITIONER

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9/PRTS

SPECIFICATION

ORGANIC ANION TRANSPORTER AND GENE CODING FOR THE SAME

TECHNICAL FIELD

The present invention is related to the genes and their encoding polypeptides, which are related to the transport of organic anions.

BACKGROUND ART

The kidney plays important roles in the excretion of endogenous compounds and xenobiotics. Anionic substances including drugs are excreted via carrier-mediated pathway(s) into the urine. The first step of this secretion is the uptake of organic anion from the peritubular plasma across the basolateral membrane of the proximal tubule cells.

The basolateral uptake of the organic anions has been studied using several techniques, such as perfusion of excised kidney, or membrane vesicles of isolated tubule cells. In these studies, para-aminohippurate (PAH) has been widely used as a test substrate. During these studies, it has been supposed that the organic anion transporter responsible for the basolateral uptake of organic anions was an organic anion/dicarboxylate exchanger.

There are, however, limitations in the previous techniques for precise analysis of the organic anions transport, such as the networks of transport between different transporters and the drug-drug interaction against a single molecule. Thus, the isolation of the organic anion transporter molecule which enables more precise analysis of the organic anion transporter has been eagerly awaited .

So far, several transporter molecules which are expressed in the liver have been isolated (Hagenbuch, B. et al. Proc. Natl. Acad. Sci. U.S.A. 88, 10629-10633, 1991, Jacquemin, E. et al. Proc. Natl. Acad. Sci. U.S.A. 91, 133-137, 1994). The cDNA cloning of organic cation transporter (OCT1), which is expressed in the kidney and the liver, was also reported (Grundemann, D. et al. Nature 372, 549-52, 1994).

As a sodium-dependent dicarboxylate cotransporter, the cDNA encoding sodium-dicarboxylate co-transporter (NaDC-1) was reported (Pajor, A.M. J. Biol. Chem. 270, 5779-5785, 1995)

Recently, OAT-K1, an isoform of oatp was isolated (Saito, H. et al. J. Biol. Chem. 271, 20719-20725, 1996). Oatp is organic anion transporting polypeptide which is expressed in the liver and mediates the sodium-independent transport of organic anions. OAT-K1 is expressed in the renal proximal tubules, however, the transport properties of OAT-K1 was distinct from that of the organic anion/dicarboxylate exchanger of the renal proximal tubule cells.

DISCLOSURE OF THE INVENTION

The aim of the present invention is to provide novel genes and the gene products, which are related to the renal transport of organic anions. The other aims of this invention will be explained in the following.

BRIEF EXPLANATIONS OF THE FIGURES

FIG. 1 shows the uptake of glutarate by the oocytes injected with rat sodium dependent dicarboxylate cotransporter (rNaDC-1) cRNA.

FIG. 2 shows the uptake experiment using the oocytes injected with rat kidney mRNA and/or rNaDC-1 cRNA.

FIG. 3 shows Hydropathy analysis of rat organic anion transporter OAT1.

FIG. 4 shows Northern blot analysis of rat organic anion transporter OAT1 using mRNAs derived form various rat tissues.

FIG. 5 shows the effect of pre-incubation with glutarate, or co-expression with rNaDC-1 was examined in oocytes injected with rat OAT1

FIG. 6 shows the effect of extracellular sodium ion on the rat OAT1-mediated uptake of PAH in oocytes injected with OAT1 cRNA.

FIG. 7 shows transport rate of different concentrations of PAH in oocytes injected with rat OAT1 cRNA was examined.

FIG. 8 shows *Cis*-inhibitory effect of various anionic substances on the rat OAT1-mediated uptake of PAH was examined.

FIG. 9 shows the result of that radio labeled drugs was examined whether they were transported by rat OAT1.

BEST MODE FOR CARRYIING OUT THE IVENTION

We isolated a novel cDNA which encodes a membrane protein, OAT1, from the rat kidney. We also isolated the human homolog of OAT1. We expressed rat and human OAT1 in the *Xenopus laevis* oocytes, and successfully demonstrated that these proteins mediated the transport of organic anions. Thus we could complete this invention.

The proteins whose amino acid sequences are described in A, B, C and D are all included in this invention.

(A) The protein whose amino acid sequence is shown in SEQUENCE No. 1.

(B) Proteins whose amino acid sequences are identical to that shown in SEQUENCE No. 1 except that several amino acid residues are deleted, substituted or added in it.

Despite of these changes, the protein must possess the ability to transport organic anions.

(C) The protein whose amino acid sequence is shown in SEQUENCE No. 2.

(D) Proteins whose amino acid sequences are identical to that shown in SEQUENCE No. 2 except that several amino acid residues are deleted, substituted or added in it. Despite of these changes, the protein must possess the ability to transport organic anions.

The DNAs whose nucleotide sequences are described in a, b, c and d are also includes in this invention.

(a) The DNA whose nucleotide sequence is shown in SEQUENCE No. 1.

(b) DNAs which can hybridize the DNA shown in SEQUENCE No. 1 in stringent condition, and encode the proteins possessing the ability to transport organic anions.

(c) The DNA whose nucleotide sequence is shown in the SEQUENCE No. 2.

(d) DNAs which can hybridize the DNA shown in SEQUENCE No. 2 in stringent condition, and encode the proteins possessing the ability to transport organic anions.

The novel protein of the present invention (OAT1: organic anion transporter 1) which possesses the ability to transport organic anions, is expressed predominantly in the renal proximal tubule cells.

The transport rate of organic anions via OAT1, i.e. the uptake rate of organic anions into the cell expressing OAT1, is stimulated by dicarboxylates present in the cells. This fact indicates that OAT1 is an organic anion/dicarboxylate exchanger. The dicarboxylates which are effluxed in exchange for organic anion via OAT1, are taken up by the sodium-dicarboxylate cotransporter from the extracellular fluid. Thus, dicarboxylate are recycled for the OAT1-mediated transport of organic anions.

The novel protein of the present invention, OAT1, possesses the ability to

transport (take up) various organic anions, such as cycic nucleotides, prostaglandins, urate, antibiotics, diuretics and anticancer drugs. Since chemical structures of these substances are diverse, the substrate selectivity of OAT1 is considered to be very wide.

The amino acid sequence of OAT1 shows no similarity to that of the previously isolated renal organic anions transporter OAT-K1. Thus, OAT1 belongs to distinct transporter family.

The SEQUENCE NO. 1 shown in the table depicts the total nucleotide sequence of rat OAT1 cDNA (approximately 2.2 kb) with the deduced amino acid sequence (551 amino acid residue) encoded by the open reading frame of rat OAT1 cDNA.

The SEQUENCE NO. 2 shown in the table depicts the total nucleotide sequence of human OAT1 cDNA (approximately 2.2 kb) with the deduced amino acid sequence (563 amino acid residue) encoded by the open reading frame of human cDNA.

We searched for the DNA database (GeneBank and EMBL) and protein database (NBRF and SWISS-PROT) for the homologues sequence of OAT1. We could not find any homologues sequences of OAT1 in the sequences whose function had been clarified.

In addition to the amino acid sequence shown in SEQUENCE NO. 1 and NO. 2, the present invention includes the following proteins. Proteins whose amino acid sequences are identical to that shown in SEQUENCE NO. 1 except that several amino acid residues are deleted, substituted or added in it. The extent of changes in amino acid sequence of these proteins are acceptable when the product proteins possess the ability to transport organic anions. Usually, numbers of the changed amino acid residues are

between one to 110, preferably 1 to 55. These amino acid sequences show 80 %, preferably 90 %, identity to that shown in SEQUENCE NO. 1 or NO. 2.

In addition to the DNAs with the nucleotide sequences shown in SEQUENCE NO. 1 and NO. 2, the present invention includes DNAs which can hybridize the cDNA shown in SEQUENCE NO. 1 and No. 2. The proteins encode by these DNAs must possess the ability to transport organic anions. Usually, these DNAs show more than 70 %, preferably 80 %, identity to those shown in SEQUENCE NO. 1 or NO. 2. These DNAs include mutated genes found in nature, artificially ? mutated genes and the genes derived from other species of living cells.

The stringent condition in hybridization screening, which we refer to in this invention, indicates that hybridization is performed at 37-42 °C for approximately 12 hours in 5 X SSC (Standard Saline Citrate) solution, or in the hybridization solution with equivalent concentrations of salts, followed by washing in 1 X SSC solution. If more high stringency condition is required, washing process can be performed in 0.1 X SSC or solutions with equivalent concentrations of salts.

The homologues genes encoding the organic anion transporter of the present invention, can be obtained from other species, such as the dogs, bovines, horses, gouts, sheep, monkeys, pigs, rabbits and mouse, using homology screening. For this purpose, cDNA library can be constructed from the kidney or culture cells of the aimed species of animals.

In addition to the homology screening, the isolation of the genes can be performed using expression cloning technique.

In the following, we will explain the method of expression cloning briefly, which we used for the isolation of the renal organic anion transporter.

mRNA (poly $(A)^+$ RNA) obtained from the rat kidney is divided into fractions

according to their size, and each fraction of mRNA is injected into Xenopus laevis oocytes with cRNA of rat sodium-dependent dicarboxylate cotransporter.

The cDNA sequence of rabbit sodium dicarboxylate cotransporter (NaDC-1) was already reported (Pajor, A.M. J. Biol. Chem. 270, 5779-5785, 1995), therefore, the cDNA of rat sodium dicarboxylate cotransporter (NaDC-1) can be easily isolated. The complementary RNA (cRNA) for rNaDC-1 cDNA can be synthesized *in vitro* using RNA polymerases, such as T3 or T7 RNA polymerase.

Oöcytes injected with rat kidney mRNA and the cRNA of rNaDC-1 are examined for the uptake rate of radio-labeled organic anions, such as PAH, and the mRNA fractions showing the highest transport rate of PAH can be determined. The cDNA library can be constructed from these selected fractions, which should contain concentrated mRNA for the PAH transporter. cRNAs can be synthesizes from the constructed cDNAs and injected into oocytes with the rNaDC-1 cRNA. By repeating the screening, the cDNA which encodes the PAH transporter can be isolated.

The sequence of the obtained clone can be determined by dideoxytermination method, and the deduced amino acid sequence encoded can be predicted.

Whether the cDNA obtained really encodes the organic anion transporter can be verified as follows. cRNA synthesized from the isolated cDNA clone is injected into Xenopus oocytes, and ability of the expressed protein to transport of organic anions can be examined as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471).

Functional analysis of the organic anion transporter, such as the exchange property of OAT1, can be examined using the oocytes expressing OAT1.

Using the cDNA of rat OAT1, homologues DNAs or chromosomal genes

derived from different tissues or different animals can be obtained from appropriate cDNA or genomic library.

Based on the sequence of this invention shown in SEQUENCE NO. 1 and NO. 2, sets of PCR (polymerase chain reaction) primers can be designed by which cDNA probes can be synthesized to search the cDNA or genomic library.

cDNA library or genomic DNA library can be constructed using methods described , for example, in "Molecular Cloning" edited by Sambrook, J., Fritsch, E.F., and Maniatis, T. Cold Spring Harbor Laboratory Press, 1989. Commercially available library can also be used.

The organic anion transporter of this invention can be produced by the molecular recombination technique. For example, the cDNA encoding the organic anion transporter is subcloned into expression vectors, followed by transformation p f appropriate host cells with them. For expression systems to produce polypeptides, host cells, such as bacteria, yeast, insect and mammalian cells can be used . Among these, insects cells and mammalian cells are preferable to obtain the proteins with functions.

When the organic anion transporter is required to be expressed in the mammalian cells, the cDNA encoding the organic anion transporter should be subcloned into mammalian expression vectors, such as retrovirus vectors, papilloma virus vectors, vaccinia virus vectors and SV40 vectors. In this case, the cDNA of organic anion transporter must be inserted after? the promoter regions, such as SV40 promoter, LTR promoter and elongation 1α promoter. Then appropriate animal cells are transformed with the recombinant vectors containing the organic anion transporter cDNA. The mammalian cells, such as COS7 cells, CHO cells, Hela cells, primary culture cells derived from the kidney, LLC-PK1 cells and OK cells, can be used for this purpose.

The cDNAs which can be used for the above mentioned purpose are not restricted to those shown in SEQUENCE NO. 1 and NO. 2. Since each amino acid is encoded by several types of codon, cDNAs which encode the proteins with the amino acid sequences shown in SEQUENCE NO. 1 and NO. 2 can be designed based on information of codons. Any codons, which encode the desired amino acid, can be selected, and cDNAs inducing more efficient expression may be designed considering the codon preference in the host cells. The designed cDNAs can be obtained by chemical DNA synthesis, digestion and ligation technique, and site-directed mutagenesis method. The methods of the site directed mutagenesis are described elsewhere (Mark, D.F., et al., Pro Nat Aca Sci, vol 81, 5662~5666, 1984)

The nucleotides which can hybridize the cDNA of OAT1 in high stringent condition can be used as probes to detect the organic anion transporters. In addition, they can be used to alter the expression level of the organic anion transporter, such as antisense-nucleotide, ribozyme and decoy. For this purpose, continuous nucleotides more than 14 base pairs, or their complementary nucleotide sequences can be used. If more specificity is required, more longer fragments, for example more than 20 to 30 nucleotides sequence, can be applied.

The antibody against the organic anion transporter of this invention can be obtained, using the fragments of the organic anion transporter or the synthesized polypeptides with the partial sequences which have equivalent immunochemical properties. Polyclonal antibody can be obtained by the ordinary immunizing method. i.e. immunize the rat or rabbit with antigen, and recover the serum. Monoclonal antibody can be obtained by the ordinary method such as hybridoma technique. These antibody can be used to detect or purify the organic anion transporter

In the following, we will explain the present invention precisely, however, this

invention is not restricted to the following description

This invention has been performed, if not indicated otherwise, using methods described in the "Molecular Cloning" (edited by Sambrook, J., Fritsch, E. F., and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989), or using commercially available reagents and kits according to the manufacturer instructions, .

EXAMPLES

EXAMPLE 1: CLONING OF RAT ORGANIC ANION TRANSPORTER

(1) cDNA cloning of rat sodium-dicarboxylate co-transporter (rNaDC-1), and the preparation of rNaDC-1 cRNA

A non-directional cDNA library was prepared from rat kidney poly(A)$^+$ RNA using commercially available kit (Superscript Choice system, GIBCO BRL) and was ligated to λZipLox EcoRI arms (GIBCO BRL). A PCR product corresponding to nucleotides 1323 1763 of the rabbit sodium dicarboxylate transporter (NaDC-1) (Pajor, A.M. (1995) J. Biol. Chem. 270, 5779-5785) was labeled with ^{32}P-dCTP. A rat cDNA library was screened with this probe at low stringency. Hybridization was done overnight in the hybridization solution at 37°C and filters were washed finally at 37°C in 0.1X SSC / 0.1% SDS. The hybridization solution contains 5 X SSC, 3 X Denhardt's solution, 0.2% SDS, 10% dextran sulfate, 50 % formamide, 0.01% Antifoam B, 0.2 mg/ml denatured salmon sperm DNA, 2.5 mM sodium pyrophosphate and 25 mM MES, pH 6.5. cDNA inserts in positive λZipLox phage were recovered in plasmid pZL1 by *in vivo* excision and further subcloned into pBluescript II SK- (Stratagene) for sequencing and *in vitro* transcription.

rNaDC-1 cRNA was synthesized *in vitro* using the rNaDC-1 cDNA as a template.

Xenopus laevis oocyte expression studies and uptake measurements were performed as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992). Defolliculated oocytes were injected with i*n vitro* transcribed cRNA of rNaDC-1, and ^{14}C-glutarate uptake was examined in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM HEPES, pH 7.4).

As shown in Fig. 1, the oocytes injected with rNaDC-1 cRNA showed the sodium-dependent uptake of glutarate, indicating that the isolated rNaDC-1 encodes the rat sodium-dependent dicarboxylate cotransporter.

(2) Cloning of the rat renal organic anion transporter OAT1.

The expression cloning of organic anion transporter 1 (OAT1) was performed using the method described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992)

Four hundreds μg of rat kidney poly(A)$^+$ RNA was size fractionated as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992) using preparative gel electrophoresis (BIO RAD, Model 491 Prep cell).

Then we co-injected poly(A)$^+$ RNAs of each fraction together with rNaDC-1 cRNA into oocytes. Before uptake study, the oocytes were routinely preincubated for two hours in ND96 solution containing 1 mM glutarate for 2 hours.

Uptake experiment was performed in oocytes injected with poly(A)$^+$ RNAs of each fraction together with rNaDC-1 cRNA. ^{14}C-PAH (50 μM) uptake was measured in ND96 solution without glutarate for 1 hour. In this experiment, only those oocytes injected with both poly(A)$^+$ RNAs of each fraction and rNaDC-1 cRNA showed

significant uptake of PAH: in contrast oocytes injected with only poly$(A)^+$ RNAs of each fraction or rNaDC-1 cRNA did not show any uptake of PAH (Fig. 2).

We determined the cRNA fractions (1.8 - 2.4 kilobase (kb) poly $(A)^+$ RNA), which induced the highest PAH uptake rate when injected with rNaDC-1 cRNA into X. oocytes. Then a directional cDNA library was constructed from these fractions using Superscript Plasmid system (GIBCO BRL), and was ligated into the Sal I and Not I site of pSPORT 1. Recombinants were electroporated into Electro Max DH10B competent cells (GIBCO BRL). Approximately 500 colonies were grown on nitrocellulose membrane. Plasmid DNA was purified from colonies of each plate. Capped cRNA was synthesized in vitro after linearization of each plasmid DNA with Not I.

Then we co-injected cRNA synthesized from each filter together with 2 ng rNaDC-1 cRNA into oocytes. When ^{14}C-PAH uptake was detected on a particular group, it was subdivided into several groups, and further screened.

After screening of eight thousands clones, we isolated a single clone (OAT1), which mediated the significant uptake of PAH.

Deleted clones obtained by Kilo-Sequence Deletion kit (Takara, Japan) or specially synthesized oligonucleotide primers were used for sequencing of OAT1 cDNA. OAT1 were sequenced by dideoxytermination method using Sequenase ver. 2.0 (Amersham) or Dye Primer Cycle Sequencing Kit (Applied Biosystems).

Then we determined the nucleotide sequence of OAT1, and deduced the coding region of OAT1 cDNA and the amino acid sequence encoded.

The nucleotide SEQUENCE NO. 1 is the sequence of OAT1

Kyte-Doolittle hydropathy analysis (Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol . 157, 105-132) of OAT1 predicts twelve putative membrane-spanning domains (Fig. 3). Five N-glycosylation sites are predicted in the first hydrophilic loop. There are

4 putative protein kinase C-dependent phosphorylation sites in the hydrophilic loop between 6 th and 7 th transmembrane domains.

(3) The tissue distribution of OAT1 analyzed by Northern blot

The tissue distribution of OAT1 mRNA was examined. Three μg of poly $(A)^+$ RNA prepared from various rat tissues were electrophoresed on a 1% agarose/formaldehyde gel and transferred to a nitrocellulose filter. The filter was hybridized at 42°C overnight in the hybridization solution with full-length OAT1 cDNA labeled with ^{32}P-dCTP. The filter was washed finally in 0.1x SSC/0.1% SDS at 65°C.

Under high stringency Northern blot analysis, a strong 2.4 kb mRNA band and two bands corresponding to longer transcripts (3.9 kb and 4.2 kb) were detected predominantly in the kidney (Fig. 4). In the kidney, expression of OAT1 mRNA is strong in the cortex and outer medulla (cortex > outer medulla) and very weak in the inner medulla.

Upon longer exposure, a faint 2.4 kb mRNA band was detected in the brain. No hybridization signals were obtained with mRNA isolated from other tissues.

(4) Intrarenal expression of OAT1 mRNA analyzed by in situ hybridization

The intrarenal expression of OAT1 was examined by *in situ* hybridization analysis. *In situ* hybridization was performed as described elsewhere (Kanai, Y. and Hediger, M.A. (1992) Nature 360, 467-471, 1992) with some modifications. Briefly, after perfusion fixation with 4% paraformaldehyde, rat kidney was excised and postfixed in 4% paraformaldehyde. Five μm cryostat sections of rat kidney were used *in situ* hybridization.

^{35}S-labeled sense and antisense cRNA were synthesized from the full-length OAT1 cDNA (in pBlueScript SK-) using T7 or T3 RNA polymerase after linearization of plasmid DNA with Spe I or Xho I, respectively. The cryosections were hybridized with the probe overnight in the hybridization solution, and washed to a final stringency of 0.1X SSC at 37°C for 30 min.

In situ hybridization of rat kidney coronal sections revealed that OAT1 mRNA is expressed in renal cortex and outer medulla, especially in the medullary rays of the cortex. Expression of OAT1 was not found in the inner medulla. This overall pattern of in situ hybridization suggests that OAT1 is most strongly expressed in the middle portion of the proximal tubule (S2).

EXAMPLE 2 : FUNCTIONAL CHARACTERIZATION OF ORGANIC ANION TRANSPORTER 1 (OAT1)

(1) The effect of the preincubation of glutarate on the transport activity of OAT1

The effect of the preincubation of glutarate was investigated in the uptake experiment using the oocytes expressed with OAT1.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 1-(2). Oocytes injected with rat OAT1 cRNA only, or both rat OAT1 and rNaDC-1 cRNA were incubated in the ND96 solution containing ^{14}C-PAH for 1 hour after preincubated them in the ND96 solution with and without 1 mM of glutarate.

Figure 5 shows the dependence of OAT1-mediated ^{14}C-PAH uptake on the intracellular dicarboxylate (glutarate) concentration. The rate of ^{14}C-PAH uptake by oocytes via OAT1 is increased by preincubation of the oocytes with 1 mM glutarate. When oocytes co-expressing rNaDC-1 and OAT1 are preincubated with glutarate,

hey showed a further increase in the rate of ^{14}C-PAH uptake. This *trans*-stimulative effect of glutarate indicates that OAT1 is an organic anion/ dicarboxylate exchanger. Control oocytes are those which were not injected with cRNA.

(2) The sodium dependency of the transport activity of OAT1

The effect of the extracellular sodium ion on the OAT1-mediated uptake of PAH was examined.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1).. In this experiment, choline 96 solution, in which 96 mM sodium chloride was replaced with equimolar of choline chloride, was also used in addition to ND96 solution.

As shown in Fig. 6, replacement of extracellular sodium with choline had no effect on the rate of ^{14}C-PAH uptake, indicating that OAT1 is a sodium independent transporter. Control oocytes were those which were not injected with cRNA.

(3) The kinetic experiment

Transport rate of different concentrations of PAH via OAT1 was measured to obtain the kinetic parameters of OAT1.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1). ^{14}C-PAH uptake was measured for 3 minutes. As shown in figure 7, OAT1-mediated PAH uptake followed Michaelis-Menten kinetics, and the estimated Km value was 14.3 ± 2.9 μM (mean ± s.e.m., N=3). This values is similar to that previously reported for the basolateral organic anion transport system (80μM) (Ullrich, K.J. and Rumrich, G. Am. J. Physiol. 254, F453-462, 1988).

(4) The substrate selectivity of OAT1 examined by inhibition study

The effect of various anionic drugs on the PAH uptake in the oocytes injected with rat OAT1 cRNA.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1). In this experiment, 2 μM of ^{14}C-PAH uptake in oocytes injected with rat OAT1 cRNA was measured in the ND96 solution with and without 2 mM of various non-labeled substances.

As shown in figure 8, cis-Inhibitory effect was observed for structurally unrelated drugs. Cephaloridine (a β-lactam antibiotic), nalidixic acid (an "old" quinolone), furosemide and ethacrynic acid (diuretics), indomethacin (a nonsteroidal anti-inflammatory drug), probenecid (an uricosuric drug) and valproic acid (an antiepileptic drug) potently inhibited (>85%) OAT1-mediated ^{14}C-PAH uptake. An antineoplastic drug, methotrexate, moderately inhibited ^{14}C-PAH uptake. Endogenous compounds, such as prostaglandin E2, cyclic-AMP, cyclic-GMP and uric acid also inhibited ^{14}C-PAH uptake.

(5) The substrate selectivity of OAT1 examined by uptake experiment using labeled anionic substances

Several radio labeled compounds were examined whether they are taken up into oocytes via OAT1.

The uptake experiment using PAH was performed as described in the methods of EXAMPLE 2-(1). In this experiment, radio labeled substances were used as substrates in stead of ^{14}C-PAH. Control oocytes were those which were not injected with cRNA.

As shown in Fig. 9, ^{3}H-methotrexate, ^{3}H-cAMP, ^{3}H-cGMP, ^{3}H-prostaglandin

E2, ^{14}C-urate and ^{14}C-α-ketoglutarate were revealed to be transported into the oocytes expressing OAT1. In contrast, any uptake of ^{14}C-TEA (tetraethylammonium: a representative organic cation) and ^{3}H-taurocholic acid were not detected (data not shown).

EXAMPLE 3 : CLONING OF THE HUMAN ORGANIC ANION TRANSPORTER

Using rat OAT1 cDNA obtained in EXAMPLE 1-(2), human cDNA library was screened. Human cDNA library was constructed from human kidney poly (A)+ RNA (Clontech).

Sequence of the isolated cDNA clone (human OAT1 cDNA) was determined according to the methods described in Example 1. The coding region of the human OAT1 cDNA and the deduce amino acid sequence was determined as well.

The sequence of human OAT1 in both nucleotide and amino acid level is shown in the SEQUENCE NO. 2.

The sequence homology between rat OAT1 and human OAT1 was approximately 85 % and 79 %, in amino acid level and nucleotide level, respectively.

INDUSTRIAL APPLICABILITY

The present invention, organic anion transporter 1 (OAT1) and the gene encoding OAT1, is considered to be useful to clarify the molecular mechanisms underlying the pharmacokinetics and toxicokinetics, such as the drug elimination and drug-drug interaction. In addition, the screening system to identify the nephrotoxic drugs and the way to protect kidney from such nephrotoxic substances will be developed, since many agents causing renal insufficiency, such as β-lactam antibiotics

and NSAIDs (non-steroidal anti inflammatory drugs), have been suggested to be transported by OAT1, and OAT1 seems to be responsible for the accumulation of these nephrotoxicants in the kidney.

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CLAIMS

1. A protein selected from the following group of A, B, C and D;

(A)a protein comprising the amino acid sequence shown in SEQUENCE No. 1,

(B) a protein comprising the amino acid sequence shown in SEQUENCE No.1 deleted, substituted or added at least one amino acid residue, and having ability to transport organic anions,

(C) a protein comprising the amino acid sequence shown in SEQUENCE No. 2, and

(D) a protein comprising the amino acid sequence shown in SEQUENCE No.2 deleted, substituted or added at least one amino acid residue, and having ability to transport organic.

2. The proteins according to claim 1, wherein said protein is derived from human.

3. The proteins according to claim 1, wherein said protein is derived from rats.

4. The protein according to claim 1, wherein said protein is derived from the kidney

5. An isolated gene encoding the protein according to claim 1.

6. An isolated gene selected from the following group of a, b, c and d;

(a) a DNA comprising nucleotide sequence shown in SEQUENCE No. 1,

(b) a DNA being able to hybridize with DNA shown in SEQUENCE No. 1 in stringent condition and encoding a protein with ability to transport organic anion,

(c) a DNA comprising nucleotide sequence shown in SEQUENCE No. 2, and

(d) a DNA being able to hybridize with DNA shown in SEQUENCE No. 2 in stringent condition and encoding a protein with ability to transport organic anion.

7. The gene according to claim 6, wherein said protein is derived from human

8. The gene according to claim 6, wherein said protein is derived from rats.

9. The gene according to claim 6, wherein said protein is derived from the kidney

10. A plasmid containing regions encoding the gene according to claims 5-9 or regions encoding the protein in said gene.

11. The plasmid according to claim 10 is expressed plasmid.

12. A host cell transformed with the plasmid according to claim 10.

13. A nucleotide comprising the partial sequence comprised of continuous at least 14 bases shown in SEQUENCES Nos. 1and 2 or complementary thereof.

14. The nucleotide according to claim 13, wherein said nucleotide is used as a probe to detect the DNA encoding protein with ability to transport organic anions.

15. The nucleotide according to claim 13, wherein is said nucleotide is used to regulate an expression of proteins with ability to transport organic anions.

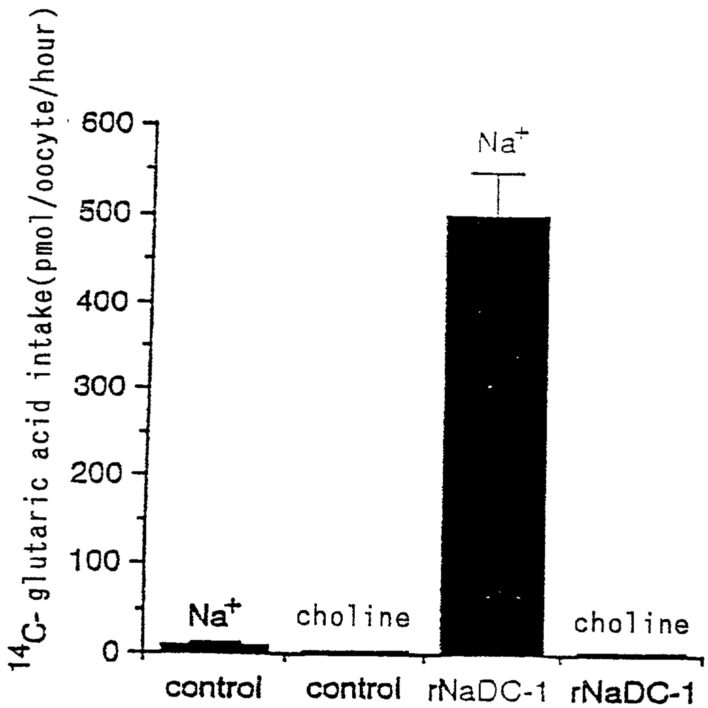
16. An antibody for the protein according to claims 1 to 4.

17. Method for screening the substrate effect of tested compound to ability of the transport of organic anions with the protein according to claims 1 to 4.

ABSTRACT

A protein capable of transporting organic anions having amino acid sequences represented by SEC ID NO: 1 or 2 or amino acid sequences derived therefrom by deletion, substitution or addition of one or more amino acid residues; and a gene coding for the protein. The protein and gene therefor are useful *in vitro* analysis of drug release and drug-drug interactions and development of methods for screening drugs useful for preventing nephrotoxicity.

figure 1



^{14}C- glutaric acid intake(pmol/oocyte/hour)
600
500
400
300
200
100
0
Na$^+$
choline
Na$^+$
choline
control
control
rNaDC-1
rNaDC-1

figure 2

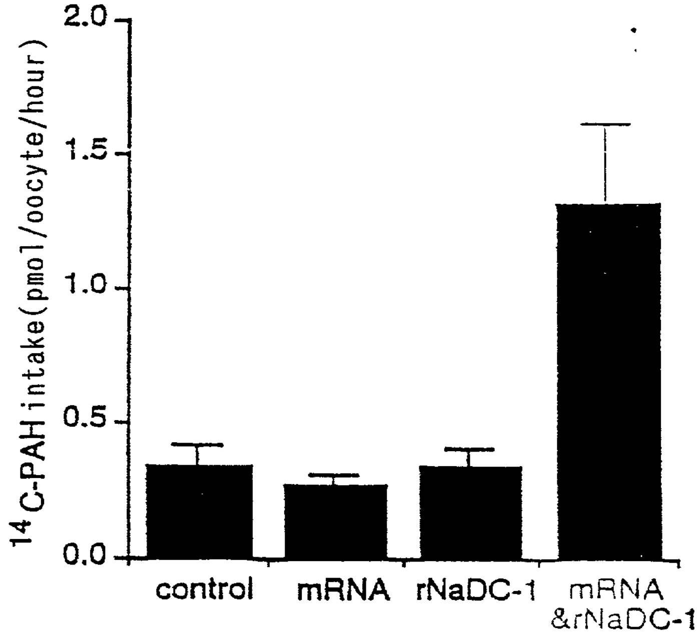
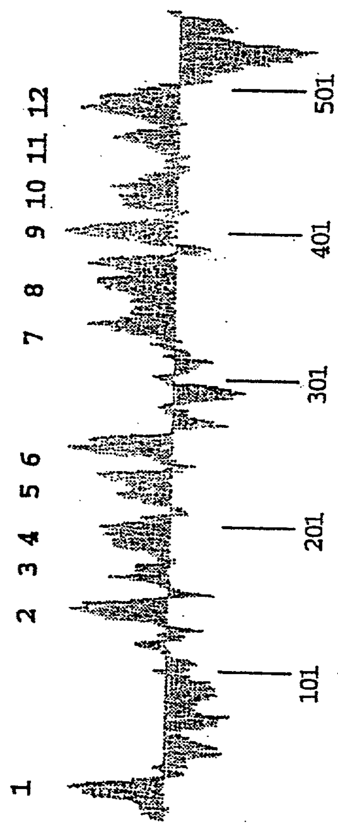

2.0
1.5
1.0
0.5
0.0
14C-PAH intake(pmol/oocyte/hour)
control
mRNA
rNaDC-1
mRNA &rNaDC-1

figure 3



OAT1

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figure 4

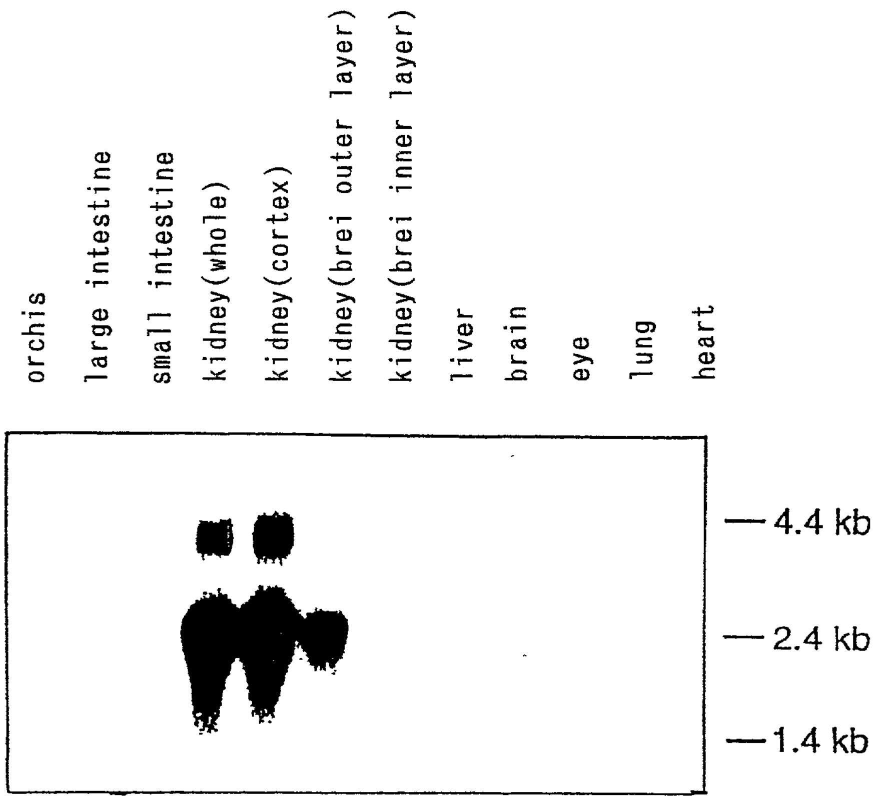


figure 5

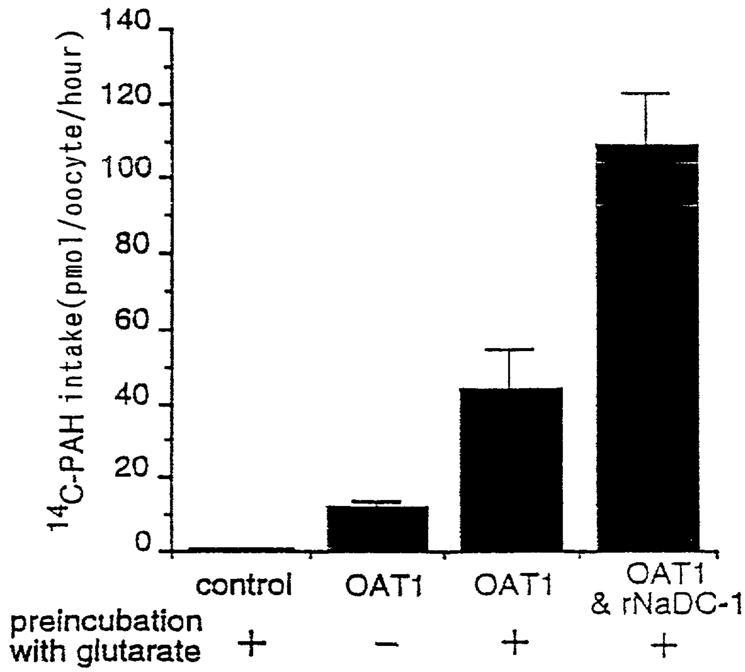
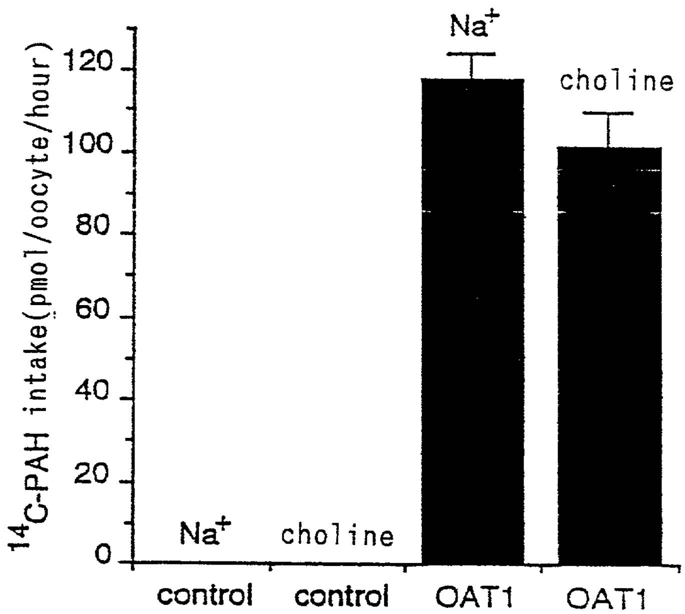
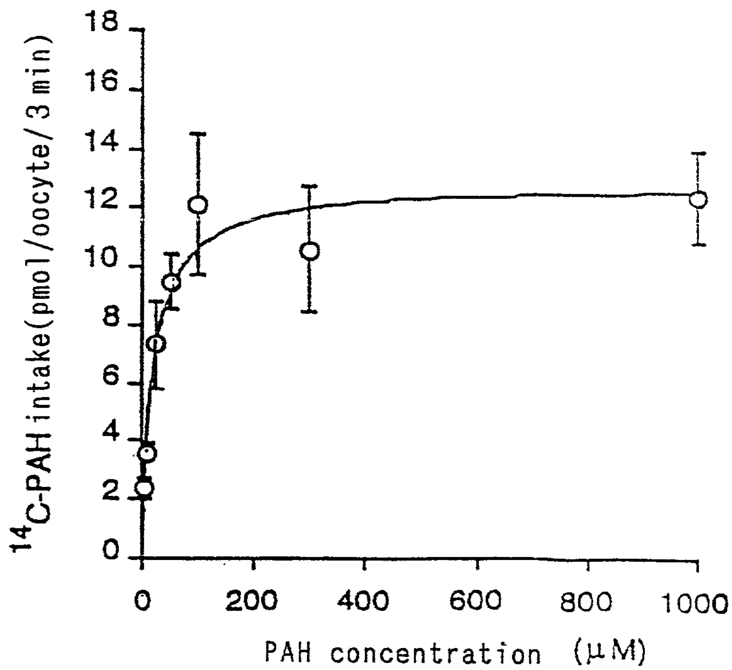

140
120
100
80
60
40
20
0
^{14}C-PAH intake(pmol/oocyte/hour)
control
OAT1
OAT1
OAT1
& rNaDC-1
preincubation
with glutarate
+
−
+
+

figure 6



^{14}C-PAH intake(pmol/oocyte/hour)
120
100
80
60
40
20
0
Na+
choline
Na+
choline
control
control
OAT1
OAT1

figure 7



^{14}C-PAH intake(pmol/oocyte/3 min)
18
16
14
12
10
8
6
4
2
0
0
200
400
600
800
1000
PAH concentration (μM)

figure 8

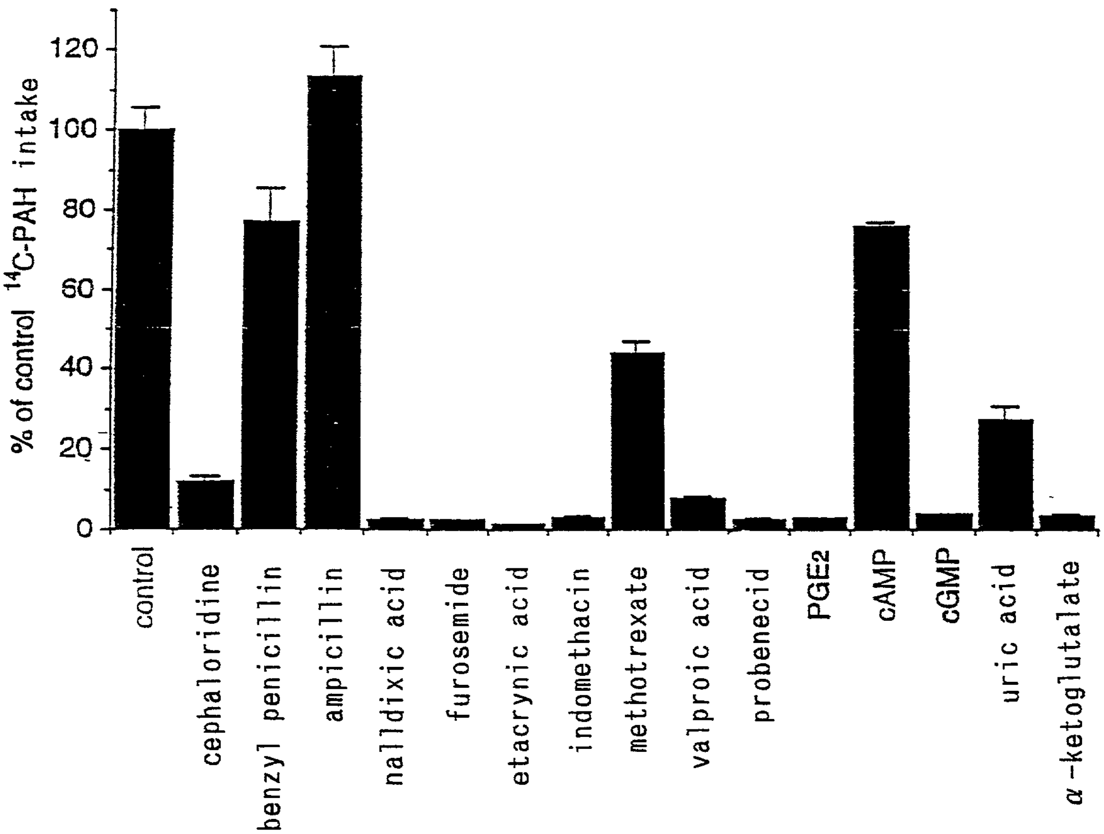
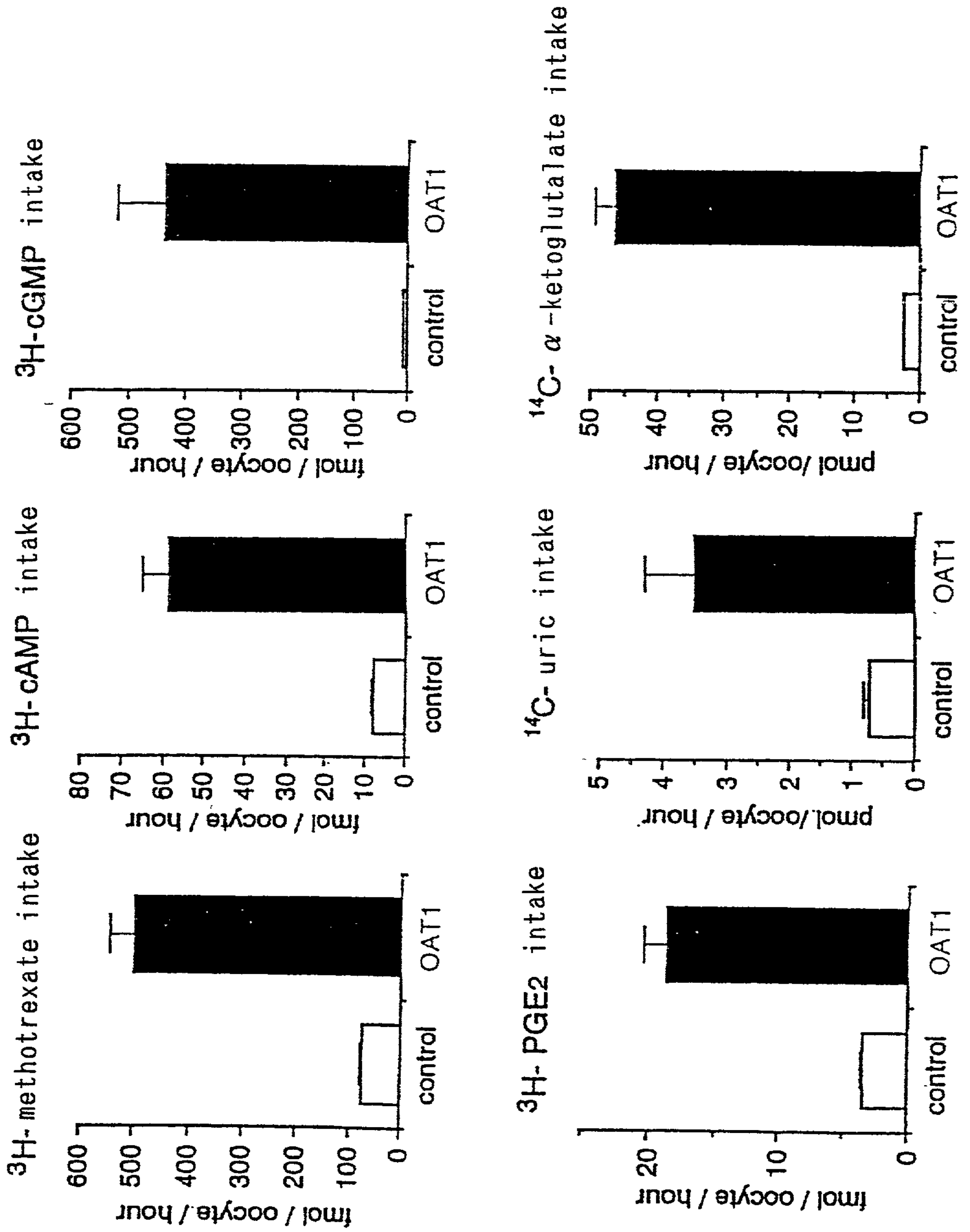

% of control 14C-PAH intake
0
20
40
60
80
100
120
control
cephaloridine
benzyl penicillin
ampicillin
nalldixic acid
furosemide
etacrynic acid
indomethacin
methotrexate
valproic acid
probenecid
PGE2
cAMP
cGMP
uric acid
α-ketoglutalate

figure 9


3H-cGMP intake
fmol / oocyte / hour
600
500
400
300
200
100
0
control
OAT1
3H-cAMP intake
fmol / oocyte / hour
80
70
60
50
40
30
20
10
0
control
OAT1
3H- methotrexate intake
fmol / oocyte / hour
600
500
400
300
200
100
0
control
OAT1
14C- α-ketoglutalate intake
pmol /oocyte / hour
50
40
30
20
10
0
control
OAT1
14C- uric intake
pmol./oocyte / hour
5
4
3
2
1
0
control
OAT1
3H- PGE2 intake
fmol / oocyte / hour
20
10
0
control
OAT1

1526/49429
PD262185

Express Mail Label No.

Docket No.
49429

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

ORGANIC ANION TRANSPORTER AND GENE CODING FOR THE SAME

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 11/22/99 as United States Application No. or PCT International Application Number 09/424,347 and was amended on ______________________

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)	Priority Not Claimed
9-134182	Japan	23 May 1997	☐
			☐
			☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
PCT/JP98/02171	18 May 1998	Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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Full name of fifth inventor, if any	
Fifth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

SEQUENCE LISTING

<110> Mr. Hitoshi ENDO

<120> ORGANIC ANION TRANSPORTER AND GENE CODING FOR THE SAME

<160> 2

<210> 1

<211> 2294

<212> DNA

<213> Rat

<223> Number of Chain : Doubled-Stranded

Topology : Linear

Kind : cDNA to mRNA

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CCTGCCACAG AACCGGCTCA GCTCCAGCTC CAGGAGTCAC TCAGCTGCAG AGGCAGTGGC   180
AGCCCCACTC CTCAGGCAAA GGGCAGCAGA CAGACAGACA GAGGTCCTAG GACTGGAGGT   240
CCTCAGTCAT TGACCACTCA GCCTGGCCCA GCCCC                              275
ATG GCC TTC AAT GAC CTC CTG AAA CAG GTG GGG GGC GTC GGA CGC         320
Met Ala Phe Asn Asp Leu Leu Lys Gln Val Gly Gly Val Gly Arg
  1               5                  10                  15
TTC CAG TTG ATC CAG GTC ACC ATG GTG GTT GCT CCC CTA CTG CTG         365
Phe Gln Leu Ile Gln Val Thr Met Val Val Ala Pro Leu Leu Leu
                 20                  25                  30
ATG GCT TCC CAC AAC ACC TTG CAG AAC TTC ACT GCC GCT ATC CCC         410
Met Ala Ser His Asn Thr Leu Gln Asn Phe Thr Ala Ala Ile Pro
                 35                  40                  45
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CCT CAT CAC TGC CGC CCA CCT GCC AAT GCC AAT CTC AGC AAA GAT 455

Pro His His Cys Arg Pro Pro Ala Asn Ala Asn Leu Ser Lys Asp

50 55 60

GGA GGT CTG GAG GCC TGG CTG CCC CTG GAC AAG CAA GGA CAA CCC 500

Gly Gly Leu Glu Ala Trp Leu Pro Leu Asp Lys Gln Gly Gln Pro

65 70 75

GAA TCG TGC CTC CGC TTT ACT TCC CCC CAG TGG GGA CCA CCC TTT 545

Glu Ser Cys Leu Arg Phe Thr Ser Pro Gln Trp Gly Pro Pro Phe

80 85 90

TAC AAT GGC ACA GAA GCC AAT GGC ACC AGA GTC ACA GAG CCC TGC 590

Tyr Asn Gly Thr Glu Ala Asn Gly Thr Arg Val Thr Glu Pro Cys

95 100 105

ATT GAT GGC TGG GTC TAT GAC AAC AGC ACC TTC CCT TCA ACC ATC 635

Ile Asp Gly Trp Val Tyr Asp Asn Ser Thr Phe Pro Ser Thr Ile

110 115 120

GTG ACT GAG TGG AAC CTT GTG TGC TCT CAT CGG GCT TTC CGC CAG 680

Val Thr Glu Trp Asn Leu Val Cys Ser His Arg Ala Phe Arg Gln

125 130 135

CTG GCC CAG TCC CTG TAC ATG GTG GGA GTG CTG CTG GGA GCC ATG 725

Leu Ala Gln Ser Leu Tyr Met Val Gly Val Leu Leu Gly Ala Met

140 145 150

GTG TTT GGC TAC CTG GCG GAC AGG CTG GGC CGC CGG AAG GTG CTG 770

Val Phe Gly Tyr Leu Ala Asp Arg Leu Gly Arg Arg Lys Val Leu

155 160 165

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Tyr Ala Pro Asn Tyr Thr Val Tyr Cys Val Phe Arg Leu Leu Ser

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Gly Met Ser Leu Ala Ser Ile Ala Ile Asn Cys Met Thr Leu Asn

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GTG GAA TGG ATG CCT ATC CAC ACC CGT GCC TAT GTG GGC ACC TTG 950

Val Glu Trp Met Pro Ile His Thr Arg Ala Tyr Val Gly Thr Leu

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ATT GGC TAT GTC TAC AGC CTG GGC CAG TTC CTC CTG GCT GGC ATC 995

Ile Gly Tyr Val Tyr Ser Leu Gly Gln Phe Leu Leu Ala Gly Ile

230 235 240

GCC TAT GCT GTG CCC CAC TGG CGC CAC CTG CAG CTT GTG GTC TCT 1040

Ala Tyr Ala Val Pro His Trp Arg His Leu Gln Leu Val Val Ser

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GTG CCT TTT TTC ATT GCC TTC ATC TAC TCT TGG TTC TTC ATT GAG 1085

Val Pro Phe Phe Ile Ala Phe Ile Tyr Ser Trp Phe Phe Ile Glu

260 265 270

TCA GCC CGC TGG TAC TCC TCC TCA GGA AGG CTG GAC CTC ACC CTC 1130

Ser Ala Arg Trp Tyr Ser Ser Ser Gly Arg Leu Asp Leu Thr Leu

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GGG GCT AAG CTA AGT ATA GAG GTG CTC CGG ACC AGC CTG CAG AAG 1220

Gly Ala Lys Leu Ser Ile Glu Val Leu Arg Thr Ser Leu Gln Lys

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GAA CTG ACT CTA AGC AAA GGC CAA GCC TCA GCC ATG GAG CTG CTG 1265

Glu Leu Thr Leu Ser Lys Gly Gln Ala Ser Ala Met Glu Leu Leu

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Trp Phe Ala Thr Ser Phe Ala Tyr Tyr Gly Leu Val Met Asp Leu

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CAG GGC TTT GGG GTC AGC ATG TAC CTT ATC CAG GTG ATT TTC GGT 1400

Gln Gly Phe Gly Val Ser Met Tyr Leu Ile Gln Val Ile Phe Gly

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GCC GTG GAC CTG CCT GCC AAG TTT GTA TGC TTC CTA GTC ATC AAC 1445

Ala Val Asp Leu Pro Ala Lys Phe Val Cys Phe Leu Val Ile Asn

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TCC ATG GGG CGC CGG CCT GCA CAG ATG GCC TCC CTG CTG CTG GCA 1490

Ser Met Gly Arg Arg Pro Ala Gln Met Ala Ser Leu Leu Leu Ala

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GGC ATC TGC ATC CTG GTG AAT GGC ATA ATA CCG AAG AGC CAT ACG 1535

Gly Ile Cys Ile Leu Val Asn Gly Ile Ile Pro Lys Ser His Thr

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ATC ATT CGC ACC TCC CTG GCT GTG CTA GGG AAG GGC TGC CTG GCT 1580

Ile Ile Arg Thr Ser Leu Ala Val Leu Gly Lys Gly Cys Leu Ala

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TCC TCT TTC AAC TGC ATC TTC CTG TAC ACC GGA GAG CTG TAC CCC 1625

Ser Ser Phe Asn Cys Ile Phe Leu Tyr Thr Gly Glu Leu Tyr Pro

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CGG GTG GGC AGC ATT GTG AGC CCG CTG GTG AGC ATG ACT GCA GAG 1715

Arg Val Gly Ser Ile Val Ser Pro Leu Val Ser Met Thr Ala Glu

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TTC TAC CCC TCC ATG CCT CTC TTC ATC TTC GGC GCT GTC CCT GTG 1760

Phe Tyr Pro Ser Met Pro Leu Phe Ile Phe Gly Ala Val Pro Val

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GTC GCC AGT GCT GTC ACT GCC CTG CTG CCA GAG ACC TTG GGC CAG 1805

Val Ala Ser Ala Val Thr Ala Leu Leu Pro Glu Thr Leu Gly Gln

500 505 510

CCG CTG CCA GAT ACA GTG CAG GAC CTG AAG AGC AGG AGC AGA GGA 1850

Pro Leu Pro Asp Thr Val Gln Asp Leu Lys Ser Arg Ser Arg Gly

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Lys Gln Asn Gln Gln Gln Gln Glu Gln Gln Lys Gln Met Met Pro

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CTC CAG GCC TCA ACA CAA GAG AAG AAT GGA CTT 1928

Leu Gln Ala Ser Thr Gln Glu Lys Asn Gly Leu

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<211> 2171

<212> DNA

<213> Human

<223> Number of Chain : Doubled-Stranded

Topology : Linear

Kind : cDNA to mRNA

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GCAGCTCGGA CTCAGCTCCC GGAGCAACCC AGCTGCGGAG GCAACGGCAG TGCTGCTCCT   180
CCAGCGAAGG ACAGCAGGCA GGCAGACAGA CAGAGGTCCT GGGACTGGAA GGCCTCAGCC   240
CCCAGCCACT GGGCTGGGCC TGGCCCA                                        267
ATG GCC TTT AAT GAC CTC CTG CAG CAG GTG GGG GGT GTC GGC CGC          312
Met Ala Phe Asn Asp Leu Leu Gln Gln Val Gly Gly Val Gly Arg
 1               5                  10                  15
TTC CAG CAG ATC CAG GTC ACC CTG GTG GTC CTC CCC CTG CTC CTG          357
Phe Gln Gln Ile Gln Val Thr Leu Val Val Leu Pro Leu Leu Leu
                20                  25                  30
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Met Ala Ser His Asn Thr Leu Gln Asn Phe Thr Ala Ala Ile Pro
                35                  40                  45
ACC CAC CAC TGC CGC CCG CCT GCC GAT GCC AAC CTC AGC AAG AAC          447
Thr His His Cys Gly Pro Pro Ala Asp Ala Asn Leu Ser Lys Asn
                50                  55                  60
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Gly Gly Leu Glu Val Trp Leu Pro Arg Asp Arg Gln Gly Gln Pro
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Glu Ser Cys Leu Arg Phe Thr Ser Pro Gln Trp Gly Leu Pro Phe
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80 85 90

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Leu Asn Gly Thr Glu Ala Asn Gly Thr Gly Ala Thr Glu Pro Cys

95 100 105

ACC GAT GGC TGG ATC TAT GAC AAC AGC ACC TTC CCA TCT ACC ATC 627

Thr Asp Gly Trp Ile Tyr Asp Asn Ser Thr Phe Pro Ser Thr Ile

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GTG ACT GAG TGG GAC CTT GTG TGC TCT CAC AGG GCC CTA CGC CAG 672

Val Thr Glu Trp Asp Leu Val Cys Ser His Arg Ala Leu Arg Gln

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CTG GCC CAG TCC TTG TAC ATG GTG GGG GTG CTG CTC GGA GCC ATG 717

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GTG TTC GGC TAC CTT GCA GAC AGG CTA GGC CGC CGG AAG GTA CTC 762

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TTC GCA CCC AAC TTC CCC ATC TAC TGC GCC TTC CGG CTC CTC TCG 852

Phe Ala Pro Asn Phe Pro Ile Tyr Cys Ala Phe Arg Leu Leu Ser

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GGC ATG GCT CTG GCT GGC ATC TCC CTC AAC TGC ATG ACA CTG AAT 897

Gly Met Ala Leu Ala Gly Ile Ser Leu Asn Cys Met Thr Leu Asn

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GCG CCT TTT TTT GCC TTC TTC ATC TAC TCC TGG TTC TTC ATT GAG 1077
Ala Pro Phe Phe Ala Phe Phe Ile Tyr Ser Trp Phe Phe Ile Glu
260 265 270

TCG GCC CGC TGG CAC TCC TCC TCC GGG AGG CTG GAC CTC ACC CTG 1122
Ser Ala Arg Trp His Ser Ser Ser Gly Arg Leu Asp Leu Thr Leu
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Arg Ala Leu Gln Arg Val Ala Arg Ile Asn Gly Lys Arg Glu Glu
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Gly Ala Lys Leu Ser Met Glu Val Leu Arg Ala Ser Leu Gln Lys
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Trp Phe Ala Thr Ser Phe Ala Tyr Tyr Gly Leu Val Met Asp Leu
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Gln Gly Phe Gly Val Ser Ile Tyr Leu Ile Gln Val Ile Phe Gly

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GCT GTG GAC CTG CCT GCC AAG CTT GTG GGC TTC CTT GTC ATC AAC 1437

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TCC CTG GGT CGC CGG CCT GCC CAG ATG GCT GCA CTG CTG CTG GCA 1482

Ser Leu Gly Arg Arg Pro Ala Gln Met Ala Ala Leu Leu Leu Ala

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GGC ATC TGC ATC CTG CTC AAT GGG GTG ATA CCC CAG GAC CAG TCC 1527

Gly Ile Cys Ile Leu Leu Asn Gly Val Ile Pro Gln Asp Gln Ser

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Ile Val Arg Thr Ser Leu Ala Val Leu Gly Lys Gly Cys Leu Ala

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Ala Ser Phe Asn Cys Ile Phe Leu Tyr Thr Gly Glu Leu Tyr Pro

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Thr Met Ile Arg Gln Thr Gly Met Gly Met Gly Ser Thr Met Ala

455 460 465

CGA GTG GGC AGC ATC GTG AGC CCA CTG GTG AGC ATG ACT GCC GAG 1707

Arg Val Gly Ser Ile Val Ser Pro Leu Val Ser Met Thr Ala Glu

470 475 480

CTC TAC CCC TCC ATG CCT CTC TTC ATC TAC GGT GCT GTT CCT GTG 1752

Leu Tyr Pro Ser Met Pro Leu Phe Ile Tyr Gly Ala Val Pro Val

485 490 495

GCC GCC AGC GCT GTC ACT GTC CTC CTG CCA GAG ACC CTG GGC CAG 1797

Ala Ala Ser Ala Val Thr Val Leu Leu Pro Glu Thr Leu Gly Gln

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CCA CTG CCA GAC ACG GTG CAG GAC CTG GAG AGC AGG TGG GCC CCC 1842

Pro Leu Pro Asp Thr Val Gln Asp Leu Glu Ser Arg Trp Ala Pro

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ACT CAG AAA GAA GCA GGG ATA TAT CCC AGG AAA GGG AAA CAG ACG 1887

Thr Gln Lys Glu Ala Gly Ile Tyr Pro Arg Lys Gly Lys Gln Thr

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